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Protection Branch Report of Test No. 3-68

Evaluation of Two NASA Biological Isolation Garments

An evaluation of two Biological Isolation Garments (BIG) 1/ submitted by NASA Manned Spacecraft Center, Houston, Texas, has been completed using aerosols of B. subtilis var niger spores. This garment is a one-piece suit fabricated from permeable "Barbac" cloth and contains a zippered closure extending diagonally from the crotch across the left chest and curving over the left ear to the top of the head (Fig. 1). A protective mask with a full-width visor is enclosed within the suit except for dual aerosol canisters which can be attached or detached from the exterior. A one-piece suit of cotton underwear is worn under the garment. The BIG will be donned by a returning lunar astronaut immediately after his exit from the space capsule in order to isolate him and thereby restrain any lunar microorganisms on his body from contaminating the earth's atmosphere. As stated in an informal communication from NASA dated 26 October 1967, the design specifications for the BIG require a minimum restraining capability of 98% of particles having a diameter of 0.45µ.

Two test methods were employed to evaluate the BIG. The standard protective clothing test method developed and used by Protection Branch quantitates the number of microorganisms penetrating a protective suit or garment from the outside to the wearer on the inside. However, because the BIG will be worn to prevent microorganisms on the subject from penetrating the suit from the inside to the outside, the reverse situation, a new method described in Phase I below, was devised to evaluate the garment simulating its actual use. As a confirmatory test, the standard method, as described in Phase II, was also used.

PHASE I.

Ten milligrams of dried <u>B. subtilis</u> var <u>niger</u> spores (6 x 10^8 spores per mg) were placed in small plastic pill vials containing five grams of diatomaceous earth. The vials were capped and the contents thoroughly mixed. The resulting mixture, containing a total of 6 x 10^9 <u>B. subtilis</u> var <u>niger</u> spores (NMD 1.8 μ) was used as the test agent in

^{*} Angelica Uniform Co., St. Louis, Mo.

the Phase I tests. Two vials were taped in an inverted position to the underwear of each of two subjects, one over the left collarbone as shown in Fig. 2, and the other to the rear of the right hip.

The BIG was then donned according to the specified NASA procedure and each subject entered a small airlock about the size of a stall shower (32 x 40 x 84 inches) (Fig. 3). Upon a signal from the test operator, the subjects flipped the caps from the plastic vials thereby releasing the bacterial spores inside their respective garment. A portable Staplex* air sampler containing Chemical Corps Type 5 filter paper as the sampling medium, located in each airlock, was immediately started and the air in each airlock was continuously sampled at a rate of 50 cfm. The sampler also serves to recirculate the air inside the test plenum. Such a high rate assured a complete sampling of the plenum air every 1.25 minutes. Prior to each test the air in the test plenum was recirculated through a collective protector (Fig. 3) containing HEPA filters to remove all the airborne organisms.

For 20 of each 30 minutes the subjects were in the airlocks, they exercised to simulate light activity. The exercise routine was performed in the following order: (1) half-knee bend; (2) raise shoulders; (3) extend each leg sideways; and (4) raise hands over head. After the test, the Type 5 paper was aseptically removed from the Staplex sampler and assayed to quantitate the number of spores collected. Because the airlocks, the BIG, and underwear were thoroughly decontaminated prior to each test, the only possible source of <u>B. subtilis</u> var <u>niger</u> spores in the airlock was from the uncapped vials inside the suit. The outward penetration was reported as the percent of the total number of spores released inside the suit which were collected on the Type 5 paper in the sampler.

PHASE II.

The standard method employed at Fort Detrick for the evaluation of protective suits uses cotton twill rectangular patches (3 cm 2 area) to sample the number of aerosolized spores deposited on the surface of the suit or on the inside clothing surfaces. These cloth patches are attached to a 3-inch strip of $\frac{1}{2}$ -inch double-coated pressure-sensitive tape which are easily attached and removed aseptically from both the

* The Staplex Co., New York, N.Y.

outside and the inside layers of test clothing. Sterile patch samplers were taped on both the underwear (Fig. 4) and BIG (Fig. 5) in the same 16 anatomical areas as diagrammed in Fig. 6.

The man chamber test facility (Fig. 7) for exposing human subjects to simulant aerosols was used for this evaluation. The subjects were exposed to an aerosol of B. subtilis var niger spores (NMD-1.2u) in the test chamber, a room 16 x 9 x 10 feet. On two opposite sides of the chamber are three small rooms that serve as airlocks, (The underwear was donned and the inner patch samplers taped in place in airlock 1. In airlock 2, the BIG was donned and the outer patch samplers were attached). The complete test facility, which also includes a shower room and dressing room, is equipped with a highly efficient filtered air circulation system that enables the exposure chamber to be maintained under reduced air pressure in relation to the airlocks. This pressurization system is designed to prevent test chamber air from escaping into the other rooms of the test facility. The system is also designed to permit one to two air changes per minute in the airlocks for rapid displacement of any biologically contaminated air with clean air.

The two test subjects remained in the chamber for one hour and performed the same exercises as in Phase I, above, during 20 minutes of this time. The temperature in the chamber ranged from 65 to 70° F and the relative humidity from 40 to 50%. Upon completion of the exposure period, they entered airlock 4 where the outer layer of patch samplers were removed. Each BIG was then carefully removed and the subjects entered airlock 5 where the inner layer of patch samplers were removed. After the subjects doffed their underwear, they showered thoroughly.

Immediately upon removal from the clothing, the patch samplers was placed in a sterile dish for transfer to the laboratory. There, each 3 cm² cloth patch was separated from the tape, placed in a known volume of sterile distilled water, and biologically assayed to determine the number of spores collected. The BIG penetration is reported on the basis of the average number of spores reaching the inner (underwear) patches as opposed to the average number collected on the outer (BIG) patches.

RESULTS AND DISCUSSION

As stated above, the BIG was designed to restrain 98% or greater (or, conversely, to permit a maximum penetration of 2%) of biological organisms having a particle size diameter of 0.45 μ during a 2-hour wearing period. Because of this specified wearing time, the mean penetration for each garment at both test conditions was extrapolated to represent a 2-hour period. As shown in Table I, the percent penetration was considerably less than 2% at both conditions using 1.8 μ and 1.2 μ particulate aerosols.

Ideally, because the BIG was specifically designed to restrain 0.45µ particles, a test organism with this particle size should have been used. However, no suitable biological simulant of this precise size is available. B. subtilis var niger was selected as the agent certain to yield the best results although the particle sizes used (NMD 1.8 μ and 1.2 μ) are slightly larger than that desired. Past test results show that when filter media are evaluated using a $l\mu$ aerosol (B. subtilis var niger spores) and a 0.1µ aerosol (Tl bacteriophage), the percent penetration difference is relatively small. For example, Harstad and Filler 2 recently reported that the comparative leakages of a glass fiber filter material when tested at a linear velocity of 5-ft per minute are 0.16% for spores and 0.47% for phage, a 3-fold difference. Therefore, if the over-all extrapolated means for the outward and inward BIG penetrations (2 hour exposure to a lu aerosol) as given in Table I, are multiplied by a factor of 3, the resulting theoretical penetrations for a 0-lu aerosol would be .855% - outward and 1.96% - inward. Because these theoretical average penetrations for a 0.1µ particle size aerosol do not exceed the specified limit of 2% penetration (98% restraining capability), it is reasonable to assume that comparable penetrations for a larger particle size, 0.45µ, will also be less than 2%. In fact the outward and inward penetration values undoubtedly would both be considerably smaller if a 0.45µ test aerosol had been used.

Although quite different test techniques were used to measure inward and outward penetration of the test garments, one a long established technique in this laboratory and the other devised for this particular test, the results were remarkably close. Since it is not too unreasonable to believe that bacterial spores would penetrate the garment in both directions with equal ease, the fact that the data

obtained using the new technique matched so closely those for the established techniques gives added confidence to them.

CONCLUSIONS

Two NASA Biological Isolation Garments (BIG) evaluated on human subjects were found to restrain more than 99% of an aerosol of B. subtilis var niger spores (NMD - 1.8 μ and 1.2 μ) as measured using two different test techniques. Data extrapolations show that the BIG would be expected to restrain more than 98% of 0.1 μ particles during a 2-hour wearing period. Therefore, values for 0.45 μ particles should fall between these percentages. Consequently we consider that the BIG meets the design specification for a restraining capability of at least 98% of 0.45 μ particles for a 2-hour period.

References

- 1. Contamination Control: Biological isolation suit for spacemen. Vol. VI, No. 11, November 1967, pp 23.
- 2. Harstad, J.B., and M.E. Filler: 1967. Evaluation of air filters with submicron viral aerosols and bacterial aerosols. Interagency Service Agreement MIPR 6.0037 with National Cancer Institute, National Institutes of Health, pp 26.

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Penetration of Two NASA Biological Isolation Garments (Test agent - B. subtilis var niger spores)

	Garment	<u> 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1</u>	Garment II	
Test No.	% Outward Penetration	% Inward Penetration	% Outward Penetration	% Inward Penetration
I	.034*	.558**	. 142*	.368**
·II	.108**	.439***	.057***	.130**
III	.186**	.299**	.132**	.167**
Meant	.241	.865	.328	. 443
Over-all Me			Inward Penetration	n654

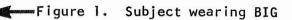
Notes:

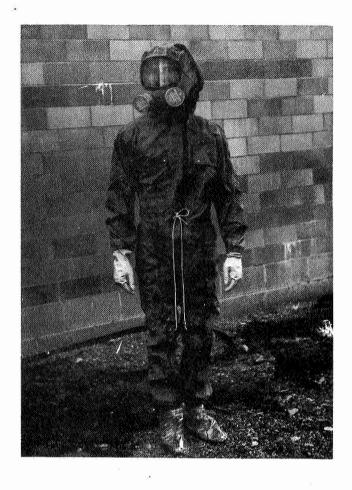
^{* 30} minute exposure

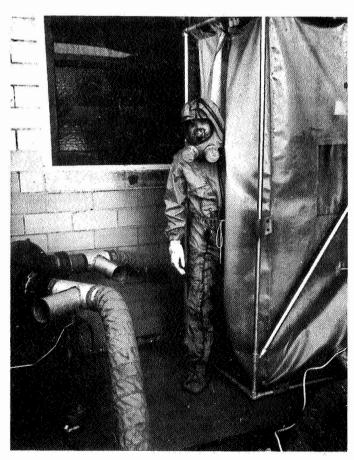
^{** 60} minute exposure

⁴⁵ minute exposure (subject forced to quit test because of breathing difficulty)

[†] Each mean was lineraly extrapolated from test results to represent a 2-hour exposure period.







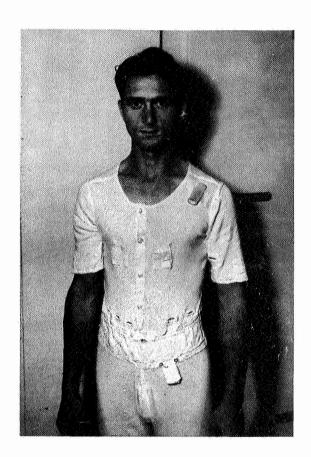


Figure 2. Subject with emptied spore dust container (after test)

Figure 3. Subject entering airlock for Phase I test

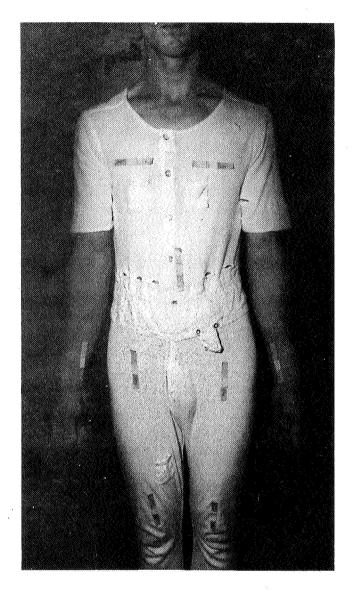


Figure 4. Subject with inner layer patch samplers



Figure 5. Subject with outer layer patch samplers

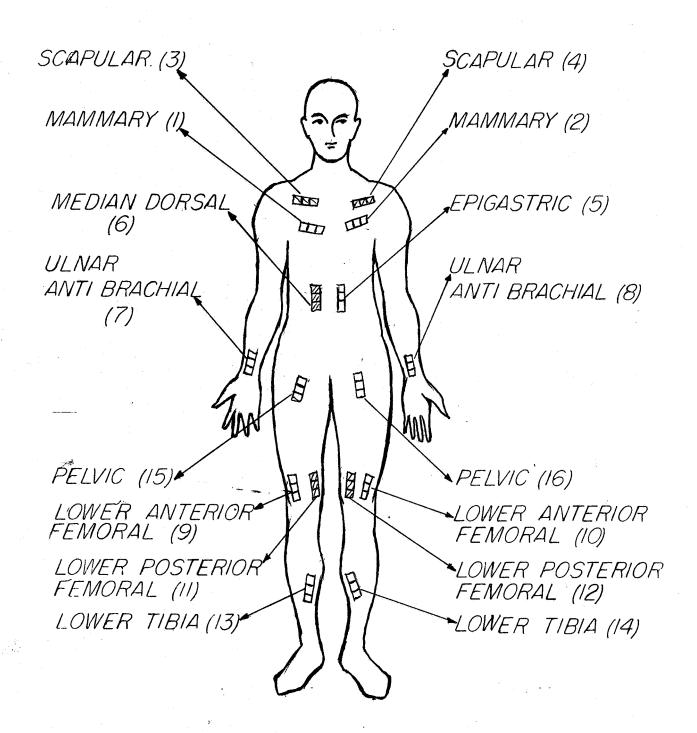


Figure 6. ANATOMICAL SAMPLING AREAS

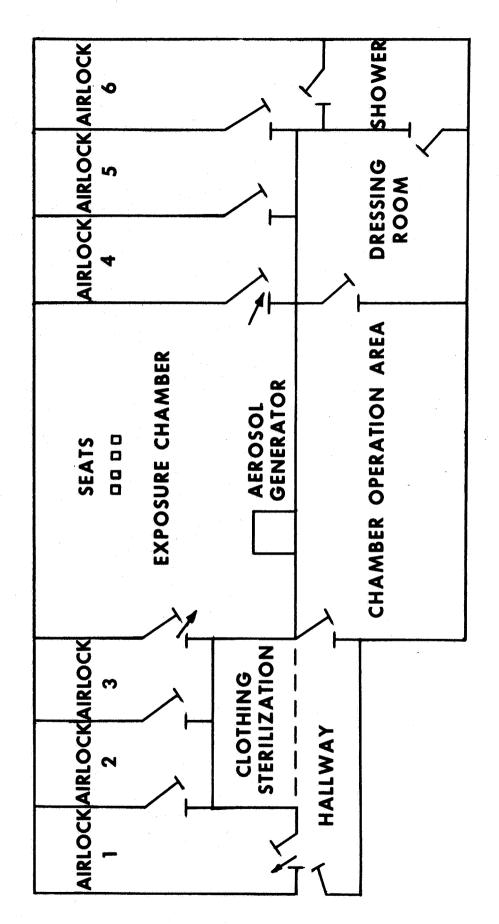


Figure 7. Man chamber test facility